TECAN.

PATENT
674182-2001

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop AF
Commissioner of Patents
P.O. 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Werner J. Hälg, declare and state that:

1. I make the declaration with U.S. application Serial No. 09/922,313. I am familiar with the prosecution history, particularly the Office Action mailed on August 8, 2005.

2. Attached is my Curriculum Vitae. In view of my education, training and experience, I consider myself qualified to express opinions stated herein.

3. The presently invention claims a method of precisely determining the volume of a dispensed liquid sample. The method relies on using a chromophoric indicator which is formed by complexing indicator ions with specific chromogenic ligands. To determine the volume of the liquid sample which is dispensed, the optical absorption of the chromophoric indicator is measured with a microplate reader. Using a standard curve, applying Beer's law and considering the measured optical density, the pipetted volumes are calculated.

4. There are a variety of methods for checking the precision of a pipetting device. The two main principles are gravimetry (pipetting onto a balance) and absorbance (using dyes and optics). Gravimetric measurements are very precise but are not amenable to multi-channel pipetting systems and cannot measure volumes below 1 µl. Absorbance measurements are used more frequently to determine the accuracy of a multi-channel pipetting device. For absorbance measurements, a standard curve is constructed by measuring the absorbance of the colorimetric reagent (dye) at various concentrations. Assuming Beer's law is followed, the standard curve (concentration vs. absorbance) will be linear. A sample whith a 'presumed' volume is then pipetted into a diluent and the absorbance is measured. From the Beer's law plot, the concentration is determined and the 'actual' volume of the pipetted sample is calculated.

5. Prior methods of performing absorbance methods to measure the accuracy of a pipetting device involve directly pipetting a given volume of a solution of the colorimetric reagent (e.g., Orange G) into a well of a microplate and than diluting the sample with a known amount of diluent. Measuring the absorbance with a microplate reader and comparing with a standard calibration curve allows determination of the accuracy of the liquid dispensing device.

6. Although prior methods are reliable for larger liquid volumes, they are not sufficient for very low volumes often used in biological assays. The essential problem with these methods is that optical absorption measurements are only accurate over a given range of absorption. If the colorimetric reagent is present in too low a concentration, the measured absorption would fall outside this range of detection. This situation occurs when measuring very low volumes in the nanoliter (nL) range where the concentration of the colorimetric reagent will be far too low, following dilution, for a reliable measurement to be made. One can potentially circumvent the problem by increasing the concentration of the colorimetric reagent such that absorption falls within the range of detection. At these high concentrations, however, the viscosity of the measured solution changes and may diverge dramatically from that of water. Moreover, many of the commonly used dyes (e.g., Orange G) stick to the walls of the pipette, particularly at high concentrations. This phenomenon has been attributed to the conjugated system in these types of dyes which strongly interact with nonpolar surfaces.

7. A goal of the present invention is to overcome the problems associated with prior methods and develop a method to determine the accuracy of liquid dispensing devices, particularly for very small volumes. Our solution was to develop a simple method where the dye can be generated *in situ* by adding a metal salt solution to a diluent containing an excess of ligands. When complexed to the metal, the initially colorless ligand becomes highly colored. Importantly, the complexation between the ligand and the metal must takes place rapidly and quantitatively. Quantitative complexation of the ligands can be assumed. E. g. the formation constant of the $[Fe(II)\text{-}(FZ)_3]^{4-}$-complex (FZ = FerroZine®) is reported as 3.39×10^{15}! (Analytical Chemistry, Vol 48, [1976] p. 1197). By pipetting the highly water soluble salt solution rather than the dye, problems with the dye sticking to the pipette surface are eliminated. Furthermore, metal salt solutions have virtually identical viscosities to that of pure water.

8. The presently claimed invention encompasses various ligands which are applicable with the methodology described in the preceding paragraphs. An objective was to find ligands that formed intensively colored, highly stable complexes when combined with metals. Because of the stability of these complexes, the reaction between the metal and the ligand will go to completion. Metals can complex with a variety of ligands to form stable colored complexes although these complexes were never used in volumetric determinations. Some of these potential complexes were investigated and a few provided satisfying results. For instance, when pipetting 10 nL volumes of a 0.25 Fe^{2+} solution to a solution of FerroZine® ligand, complexes with a deep violet color are formed which have a molar absorptivity exceeding 22,000. Examining Beer's law, one observes that only minute quantities of the ligand need be present in order to obtain optical measurements of the absorption. Therefore, the properties of the water (e.g. viscosity) will not be affected when the dye forms and the standard Beer's law curve can be readily applied, even when using rather concentrated salt solutions to measure extremely small volumes.

9. The methodology can be readily extended to non-aqueous systems including dimethylsulfoxide (DMSO).

10. The Examiner rejects many of the claims under 35 U.S.C. 103(a) as being unpatentable over U.S. patent No. 5,0631,639 to Lung et al in view of U.S. patent No. 5,329,969 to Bauer et al. Neither of these patents, alone or taken together, suggest the invention in the present application.

11. The Examiner alleges that Bauer "teaches a reagent composition comprising a complex formed from a polyvalent metal ion having a valence of at least two and an indicator capable of interacting with the metal ion to provide a polyvalent metal-ion indicator." As stated above, it was known that metal ions complex with ligands, an observation made long before the publication of Bauer. Bauer does not apply the colored complexes to accurately measure volumes. Instead, the complexes are used for determinations of specific gravity of a test sample, such as urine. To do so, Bauer merely recites that metal ions in urine samples will displace a polyvalent metal ion from the indicator molecule resulting in a color change. The extent of the color change is used to determine the specific gravity of the urine. The technique bears no resemblance to the present invention and provides no motivation to use it in volumetric

determinations. Volumetric determinations will necessarily rely on utilizing optical absorption measurements. Bauer relies only on color changes of a sample. Hence, the advantages of the present invention, such as using very low concentrations of the indicator to make precise measurements, bear no relationship to the technique taught in Bauer.

12. The Examiner also states that Bauer teaches using metal ions and indicators that are used in the present application. Although some of the ligands are mentioned in Bauer, it should be noted that Bauer cites every known indicator that binds to a polyvalent metal ion as examples of potential colorimetric reagents (see e.g., col. 12, line 54 through col. 13, line 20). In addition, there is no actual preference by Bauer that would direct a reader to the indicator molecules claimed in the present application. Based on the patent specification of Bauer, one would assume that all of these indicators behave in an identical fashion. In reality, of the plethora of examples given, only a small fraction would display the extinction coefficients of the complexes described in the present invention. However, there is absolutely no indication of these compounds in Bauer. In actuality, the expressly pointed out examples for indicator molecules are all planar complexes with metal ions. Unlike the indicators used in the present invention, these have much lower extinction coefficients and tend to stick to the walls of a pipetter, which results in erroneous volume determinations.

13. Lung relates to a variation of the classical method of making volumetric determinations described above. In Lung, The dispensed volume to be measured is a solution of a colorimetric reagent such as cobalt sulfate. A known volume (e.g. 100 μL) of the colorimetric solution is placed in a well by reference pipette. A desired volume (e.g. 10 μL) of the colorimetric reagent is placed in a second well containing the diluent. Assuming that the absorbance of the colorimetric reagent is proportional to its concentration (Beers Law), the actual volume of the liquid placed in the second well can be calculated by comparing the absorbance in each well. The advantage of this method in Lung is that the actual concentration of the colorimetric reagent need not be known precisely.

14. Lung differs considerably from the invention disclosed in the present application. Lung requires that the experimenter use two wells for each volume being determined. Although this may be amenable to sophisticated liquid dispensing system in Lung, it adds another potential source of error in performing the experiments. Notably, the methodology described in Lung can

not be applied to the measurement of very small volumes. The Examiner states that "it would have been obvious to one of ordinary skill in the art to substitute the colorimetric reagent of Lung for the polyvalent metal ion-indicator complex of Bauer et al to provide a reagent resulting in enhanced color transition." It is respectfully pointed out that only in hindsight, one could construe that the skilled person would consult Bauer because of the coincidence of the indicator molecules cited. However, even if one were to undertake the laborious task of going through the thousands of possible polyvalent ion/ligand combinations, find one with the tremendous extinction coefficient disclosed in the present application and then apply the resulting reagent to Lung's system, the technique would still be considerably different than the present invention. Lung does not suggest forming the colorimetric reagent *in situ* and does not contemplate a method of measuring very small volumes.

15. The Examiner apparently believes that besides using different colorimetric reagents, the only difference between the present invention and Lung is the sequence in which the reagents are added. However, any mention of sequencing is irrelevant because the techniques are substantially different. The confusion may be the result of some ambiguities in the patent specification of Lung. The Examiner makes the following statement with respect to Lung: "In other words, a portion of the colorimetric reagent is separated from the first well and introduced in a second well." Actually, this is not the sequence of events in the method of Lung. After the first well is filled with the colorimetric reagent by a reference pipette, a portion from the first well is not taken out and placed in the second well. Instead, the colorimetric solution added to the second well comes from the same stock solution added to the first well. The technique differs immensely from the present application where the metal salt solution must be added to the solution containing a ligand in order to form the colored complex.

16. Based upon the above statements, neither U.S. patent No. 5,329,969 to Bauer nor U.S. patent No. 5,0631,639 to Lung, alone or in combination, teach or suggest the invention in the present application.

17. All statements made herein are of my own knowledge are true and all statements on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 5-Jan-2006

Dr. Werner J. Hälg

Curriculum Vitae

Dr. Werner J. Hälg

Oct. 2004 to present	Senior Liquid Handling Scientist in the Technology Development Group of Tecan
May 2001 to present	Corporate IP Coordinator for Tecan Group
Nov. 2000 to Oct. 2004	Deputy of Project Leader of a Project for the development of a 384 channel pipetting head for volumes down to 200 nl
Sep. 1997 to Oct. 2000	Liquid Handling Scientist in the NanoPipetting project team. Development of a method for determining the volume of small liquid samples within the project
Apr. 1996 to Sep. 1997	Liquid Handling Scientist in several projects
Apr. 1996	Joining Tecan
Sep. 1994 to Mar. 1996	Teaching assistant for undergraduate students in pharmaceutical sciences at ETH Zürich (Swiss Federal Institute of Technology)
Oct. 1988 to Aug. 1994	Ph. D. Thesis at ETH Zürich (Swiss Federal Institute of Technology) in metal-organic chemistry (homogeneous catalysis with chiral metal complexes)
Apr. 1988 to Sep. 1988	carrying out a placement at Central Research Laboratories of Ciba-Geigy, Basle
Nov. 1983 to Apr. 1988	Graduated Studies in Chemistry at ETH Zürich (Swiss Federal Institute of Technology) with diploma (Dipl. Chem. ETH)